Size, Water and Energy Content of Eggs of the Freshwater Turtles, *Emydura signata* and *Chelodina expansa*

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The relationships between egg dimensions, fresh egg mass, eggshell mass, fractional water content of egg contents and energy density of dry egg contents were determined for two species of Australian freshwater turtles, the Brisbane river turtle (*Emydura signata*) and the Broad-shelled river turtle (*Chelodina expansa*). Egg mass varied between 4.7 g and 10.6 g for *E. signata* and 10.4 g to 24.9 g for *C. expansa*. Egg length and width were good predictors of fresh egg mass in both species. Shell mass averaged 12% of fresh egg mass but varied significantly with fresh egg mass from 13.2% in 5 g eggs to 11.4% in 10.5 g eggs in *E. signata*, and averaged 14.6% over the entire egg size range in *C. expansa*. Fractional water content of fresh egg contents averaged 80.1% over the entire egg size range in *E. signata*, and averaged 78% but varied significantly from 81.8% in 10.5 g eggs to 73.3% in 24 g eggs in *C. expansa*. Energy density of dried egg contents did not vary significantly over the entire egg size range in either species and averaged 25.05 kJ/g in *E. signata* and 26.21 kJ/g in *C. expansa*.

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INTRODUCTION

Reproduction is fundamental to the persistence of all organisms. Consequently many biologists are interested in the effort made by organisms to reproduce themselves. One approach to this field of research is to measure or estimate the energy expended by organisms during reproduction. In animals this can be quite a complex process because energy may be expended in defending territory, obtaining mates and caring for young as well as producing eggs. In oviparous reptiles such as turtles, a good index of energy expended on reproduction is the energy content of eggs within a clutch, because energy expenditure obtaining mates is thought to be small and there is no post-oviposition expenditure of energy (Congdon and Tinkle 1982, Congdon et al. 1983, Booth 1998).

The most accurate way to estimate clutch energy expenditure is to measure the energy content of eggs by bomb calorimetry. This is necessarily an invasive and destructive process and therefore not a viable method if the reproductive effort of entire populations is the focus of interest, as is frequently the case in ecological studies. However, if the relationship between egg size and energy content can be quantified for a species, then only egg size and clutch size are needed to estimate reproductive effort. In freshwater turtles, egg size and clutch size can be accurately determined by X-ray radiography of gravid females with oviducal eggs (e.g. Gibbons and Greene 1979, Congdon et al. 1983, Dodd 1997, Hintow et al. 1997). After X-ray radiography, females are returned intact to their place of capture. This relatively uninvasive method can be used to estimate the reproductive effort of a population without the need for destructive sampling once the relationship between egg dimensions and egg energy content is determined. In this paper I present data on the relationships between egg dimensions (which can be measured from X-ray radiographs), egg mass, and egg water and energy content for two species of Australian freshwater turtles, the Brisbane river turtle (*Emydura signata*) and the broad-shelled river turtle (*Chelodina expansa*).

MATERIALS AND METHODS

Clutches of *E. signata* and *C. expansa* eggs were collected from breeding sites located around the University of Queensland St. Lucia campus waste water treatment ponds near Brisbane, Australia (27°32′S, 153°00′E) between 1994 and 1997. Daily searching of oviposition areas insured clutches were less than 24 h old when collected. These ponds were constructed in 1981 and colonized by turtles soon after construction. The source of colonizing animals is not known, but both species occur naturally in streams and ponds in the Brisbane area (Cann 1998, D. Booth pers. obs.). Both species emerge to oviposit after rain, *E. signata* between October and January, and *C. expansa* between March and June. Eggs were collected from nests and transported to the laboratory in moist paper towel lined plastic containers within 30 min of collection. Once in the laboratory, eggs were rinsed with tap water to remove any adhering soil and blotted dry with paper towel. Eggs were then weighed (±0.1 mg) with an electronic balance and their length and width measured (±0.1 mm) with calipers.

Samples of 2–4 eggs from 16 clutches of *C. expansa* and 8 clutches of *E. signata* were used for analysis of water and energy content. Eggs were cut open with scissors and the mass of the wet contents recorded. No attempt was made to separate the yolk from the albumen. The eggshell including the attached membrane was blotted dry and weighed. The contents were dried to constant mass at 60°C to determine water content and stored frozen at –20°C until bomb calorimetry was performed. For bomb calorimetry, samples were thawed, ground to a paste with a mortar and pestle, and then placed in an oven at 60°C for 24 h. From each sample 3 sub-samples (0.2–0.5 g) were ignited in an adiabatic bomb calorimeter (Gallenkamp auto bomb, England) previously calibrated by igniting pre-weighed samples of thermochemical standard benzoic acid (26.442 kJ/g). In all cases the 3 sub-samples gave energy densities within 2% of each other, so an average was calculated from all three values and this assigned to the egg from which the sub-samples were obtained.

Linear regression, multiple regression and Pearson product moment correlation analysis were used to determine descriptive relationships between fresh egg mass (FEM) and other variables. When analyzing the relationship between FEM and egg energy content, water content and eggshell mass, a single value was used for each clutch, and this valve was derived from the average for all eggs sampled within the clutch. Results are presented as means ± standard errors of the mean. Energy density of egg contents was compared between *E. signata* and *C. expansa* using a Mann-Whitney Rank Sum test.

RESULTS

Emydura signata

A total of 385 *E. signata* eggs from 21 clutches were collected (Table 1). Eggs were oval in shape with egg length (EL) always exceeding egg width (EW). Multiple regression analysis indicated that EL (mm) and EW (mm) together were better predictors of FEM (g) (Eq. 1) than either EL or EW alone (Eqs. 2 and 3). EL was a slightly better predictor (as indicated by a higher R^2 value) of FEM than EW.

Eq. 1:
$$FEM = EL \times 0.3232 + EW \times 0.5471 - 13.532$$
$R^2 = 0.98$, $P < 0.001$, $N = 385$

Eq. 2:
$$FEM = EL \times 0.5545 - 10.040$$
$R^2 = 0.90$, $P < 0.001$, $N = 385$

Eq. 3:
$$FEM = EW \times 1.054 - 13.291$$
$R^2 = 0.88$, $P < 0.001$, $N = 385$

Water content of egg contents was independent of FEM (Pearson correlation: $R^2 = 0.07$, $P = 0.52$, $N = 8$) and averaged 80.1 ± 0.5%. Energy density of dry egg contents was independent of FEM (Pearson correlation: $R^2 = 0.27$, $P = 0.27$, $N = 8$) and averaged 25.05 ± 0.08 kJ/g. The fractional mass of the eggshell varied significantly with FEM with small eggs having proportionately more eggshell (Fig. 1). Egg energy content can be accurately predicted from FEM (Fig. 2).

TABLE 1.

Egg and clutch characteristics for *E. signata* and *C. expansa*.

Parameter	*E. signata*	*C. expansa*
clutch sample size	21	24
mean clutch size	18.3	15.0
clutch size standard error	0.9	0.7
clutch size range	9–25	9–23
egg sample size	385	359
mean egg mass (g)	7.67	16.51
egg mass standard error (g)	0.08	0.18
egg mass range (g)	4.69–10.57	10.39–24.92
mean egg length (mm)	31.9	38.5
egg length standard error (mm)	0.1	0.2
egg length range (mm)	25.6–37.7	32.0–45.2
mean egg width (mm)	19.9	27.0
egg width standard error (mm)	0.1	0.1
egg width range (mm)	16.4–22.3	23.4–30.8

Chelodina expansa

A total of 359 *C. expansa* eggs from 24 clutches were collected (Table 1). Eggs were oval in shape with egg length always exceeding egg width. Multiple regression analysis indicated that EL and EW together were better predictors of FEM (Eq. 4) than either EL or EW alone (Eqs. 5 and 6). EW was a better predictor of FEM than EL.

Eq. 4:
$$FEM = EL \times 0.4883 + EW \times 1.135 - 32.956$$
$R^2 = 0.99$, $P < 0.001$, $N = 359$

Eq. 5:
$$FEM = EL \times 1.044 - 23.713$$
$R^2 = 0.83$, $P < 0.001$, $N = 359$

Eq. 6:
$$FEM = EW \times 1.724 - 30.016$$
$R^2 = 0.91$, $P < 0.001$, $N = 359$

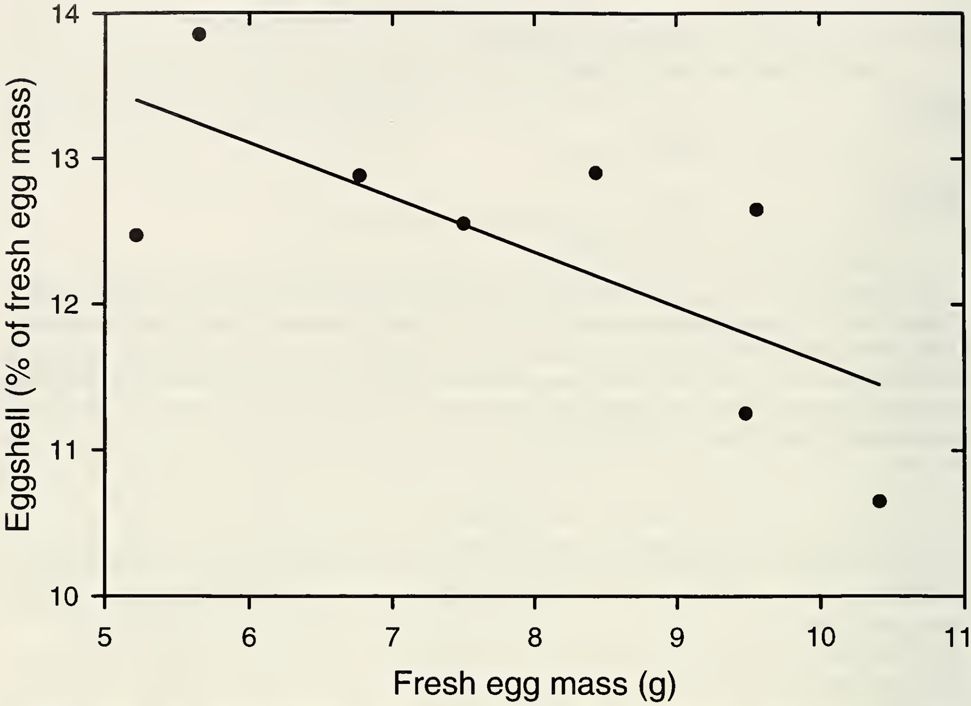

Figure 1. Relationship between eggshell mass as a percentage of fresh egg mass and fresh egg mass for *E. signata eggs*. Line is the least squares linear regression:
Eggshell mass (as % fresh egg mass) = 15.359 – FEM (g) x 0.3756
$R^2 = 0.51$, P = 0.05, N = 8.

Water content of egg contents varied significantly with FEM (Fig. 3). Energy density of dry egg contents was independent of FEM (Pearson correlation: $R^2 = 0.03$, P = 0.63, N = 16) and averaged 26.21 ± 0.03 kJ/g. The fractional mass of the eggshell was independent of FEM (Pearson correlation: $R^2 = 0.09$, P = 0.25, N = 16) and averaged 14.6 ± 0.4%. Egg energy content can be accurately predicted from FEM (Fig. 2).

DISCUSSION

By combining the relationships in Eqs. 1, 4 and figure 2 energy content of *E. signata* and *C. expansa* eggs can be reliably predicted from measurement of EL and EW.

For *E. signata*: Energy content (kJ) = EL (mm) x 1.551 + EW (mm) x 2.626 – 68.563

For *C. expansa*: Energy content (kJ) = EL (mm) x 3.645 + EW (mm) x 8.472 – 287.068

EL and EW can be accurately measured from X-ray radiographs if a known length metal object is placed beside the female turtle when she is being radiographed, and this measurement used to correct for the magnifying effect usually present in such radiographs. Hence X-ray radiographs of gravid females is a relatively uninvasive method to provide accurate data on reproductive energy expenditure in these two turtle species.

EW and EL alone were good predictors of FEM in both *E. signata* and *C. expansa*. In *C. expansa* EW was a slightly better predictor than EL, a finding consistent with studies of North American freshwater turtles (Congdon and Tinkle 1982,

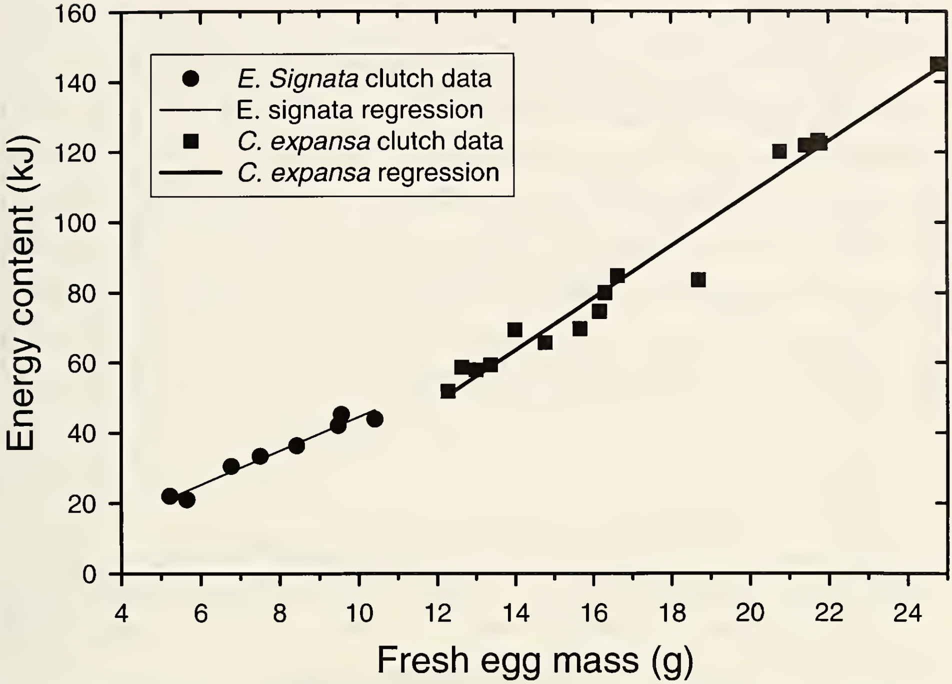

Figure 2. Relationship between egg energy content against fresh egg mass for *E. signata* and *C. expansa eggs.* Data points are mean values for eggs sampled from different clutches. Least squares linear regression for *E. signata* eggs:
Energy content (kJ) = 4.800 x FEM (g) – 3.609
$R^2 = 0.96$, $P < 0.001$, $N = 8$
Least squares linear regression for *C. expansa* eggs:
Energy content (kJ) = 7.464 x FEM (g) – 41.084
$R^2 = 0.97$, $P < 0.001$, $N = 16$

Schwarkopf and Brooks 1986, Iverson 1991, Iverson and Ewert 1991, Iverson and Smith 1993, Rowe 1994). In contrast, EL was as good a predictor of FEM as EW in *E. signata.*

Eggshell mass was a constant fraction (14.6%) of FEM over the entire egg size range in *C. expansa* but smaller eggs had proportionately heavier eggshells than larger eggs in *E. signata. C. expansa* eggs are generally larger than *E. signata* eggs (Table 1, Fig.2). However in eggs of the overlapping size range (10–11 g), *C. expansa* have a heavier eggshell and thus lighter egg contents compared to *E. signata* eggs. The relative eggshell mass of both *E. signata* and *C. expansa* eggs is lighter than the hard-shelled eggs of pig-nosed turtle *Carettochelys insculpta* (16.5%) which have a FEM averaging 34 g (Webb et al. 1986).

Fractional water content of the contents of fresh *E. signata* eggs varied around a mean of 80.1% over the entire egg size range. In contrast, egg contents of smaller *C. expansa* eggs had a higher fractional water content than large eggs (Fig. 3). This might be explained by smaller eggs having a higher albumen to yolk ratio than larger eggs (Booth 1998) because albumen has a higher water content than yolk. Interestingly, the fractional water contents of both *E. signata* and *C. expansa* eggs are greater than those found in 12 species of fresh water turtles than lay parchment shelled eggs (mean 69%, range 61%–73%) examined by Congdon and Gibbons (1985) but similar to the water content of the hard shelled eggs laid by the turtle *Trionyx triunguis* (78.6%, Leshem et al. 1991). Thus the ratio of albumen to yolk may also differ markedly between fresh water turtle

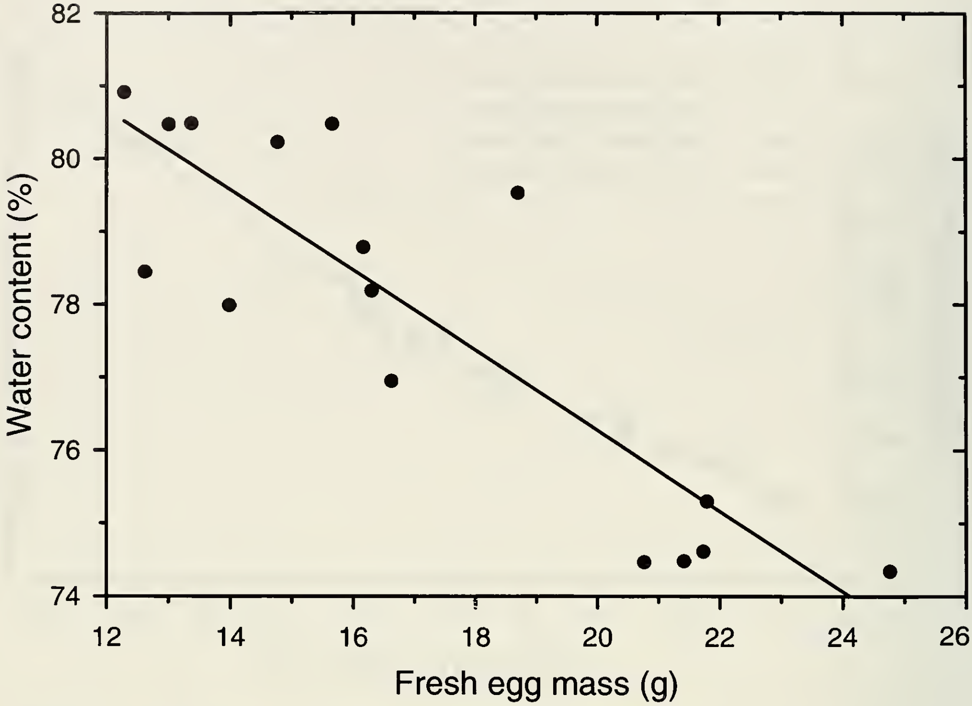

Figure 3. Relationship between percent water content of fresh egg contents and fresh egg mass for *C. expansa*. Line is least squares linear:
Water content of contents (%) = 87.277 – fresh egg mass (g) x 0.5506
$R^2 = 0.75$, $P < 0.001$, $N = 16$

species that lay hard and pliable shelled eggs. The biological significance of such a difference is obscure, but may reflect differences in incubation strategy, as the amount of water absorbed from or lost to the environment affects hatchling size in species that lay pliable shelled eggs (e.g. Gutzke et al. 1987, Miller 1993, Packard 1999) but does not affect hatchling size in species that lay hard shelled eggs (Martin 1999, Packard 1999).

The energy density (on a dry weight basis) of both *E. signata* and *C. expansa* egg contents was independent of FEM, but was significantly ($P < 0.001$) greater in *C. expansa* (26.21 kJ/g) compared to *E. signata* (25.05 kJ/g). This reflects a slightly higher lipid to protein ratio of *C. expansa* egg contents (D. Booth pers. data) as lipid has a higher energy density than protein.

Data on the energy density of dry contents of freshly laid freshwater turtle eggs are scarce. Values have been reported for *Chyrsemys picta* (26.4 kJ/g, Congdon and Tinkle 1982), *Chelydra serpentina* (28.5 kJ/g, Wilhoft 1986) and *Trionyx triunguis* (28.5 kJ/g, Leshem et al. 1991). These values are slightly higher than those for *E. signata and C. expansa* reported here. However, more data from other species are needed before any generalisations about egg contents energy density of freshwater turtles can be made.

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REFERENCES

Booth, D.T. (1998). Egg size, clutch size, and reproductive effort of the Australian broad-shelled river turtle, *Chelodina expansa. Journal of Herpetology* **32**, 592–596.

Cann, J. (1998). Australian Freshwater turtles. Beaumont Publishing, Singapore. 292 pp.

Congdon, J.D. and Gibbons, J.W. (1985). Egg components and reproductive characteristics of turtles: relationship to body size. *Herpetologica 41*, 194–205.

Congdon, J.D., Gibbons, J.W. and Greene, J.L. (1983). Parental investment in the chicken turtle (*Deirochelys reticularia*). *Ecology* **64**, 419–425.

Congdon, J.D. and Tinkle, D.W. (1982). Reproductive energetics of the painted turtle (*Chrysemys picta*). *Herpetologica* **38**, 228–237.

Dodd, C.K. (1997). Clutch size and frequency in Florida box turtles (*Terrapene carlina bauri*): implications for conservation. *Chelonian Conservation and Biology* **2**, 370–377.

Gibbons, J.W. and Greene, J.L. (1979). X-ray photography: a technique to determine reproductive patterns of freshwater turtles. *Herpetologica* **35**, 86–89.

Gutzke, W.H., Packard, G.C., Packard, M.J. and Boardman, T.J. (1987). Influence of hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). *Herpetologica* **43**, 393–404.

Hintow, T.G., Fleddermann, P.D., Loveridge, J.E., Congdon, J.D. and Gibbons, W.J. (1997). Radiographic determination of fecundity: is the technique safe for developing turtle embryos? *Chelonian Conservation and Biology* **2**, 409–414.

Iverson, J.B. (1991). Life history and demography of the yellow mud turtle, *Kinosternon flavescens. Herpetologica* **47**, 373–395.

Iverson, J.B. and Ewart, M.A. (1991). Physical characteristics of reptile eggs and a comparison with avian eggs. In 'Egg icubation: its effects on embryonic development in birds and reptiles' (Eds D.C. Deeming and M.W.J. Ferguson) pp. 88–100. (Cambridge University Press: New York).

Iverson, J.B. and Smith, G.R. (1993). Reproductive ecology of the painted turtle, (*Chrysemys picta*) in the Nebraska sandhills. *Herpetologica* **47**, 371–393.

Leshem, A., Ar. A. and Ackerman, R.A. (1991). Growth, water, and energy metabolism of the soft-shelled turtle (*Trionyx triunguis*) embryo: effects of temperature. *Physiological Zoology* **64**, 568–594.

Martin, K. (1999). The effect of temperature and water potential on the Brisbane river turtle, *Emydura signata*: embryonic development, hatchling morphology and locomotor performance. Unpublished Honours Thesis, Department of Zoology and Entomology, The University of Queensland.

Miller, K. (1993). The improved performance of snapping turtles (*Chelydra serpentina*) hatched from eggs incubated on wet substrate persists through the neonatal period. *Journal of Herpetology* **27**, 228–233.

Packard, G.C. (1999). Water relations of Chelonian eggs and embryos: is wetter better? *American Zoologists* **39**, 289–303.

Rowe, J.W. (1994). Egg size and shape variation within and among Nebraskan painted turtle (*Chrysemys picta belli*) populations: relationships to clutch and maternal body size. *Copeia* **1994**, 1034–1040.

Schwarzkopf, L. and Brooks, R.J. (1986). Annual variations in reproductive characterisitics of painted turtles (*Chrysemys picta*). *Canadian Journal of Zoology* **64**, 1148–1151.

Webb, G.J.W., Choquenot, D., and Whitehead, P.J. (1986). Nests, eggs, and embryonic development of *Carettochelys insculpta* (Chelonia: Carettochelidae) from Northern Australia. *Journal of Zoology London (B)* **1**, 521–550.

Wilhoft, D.C. (1986). Eggs and hatchling components of the snapping turtle (*Chelydra serpentina*). *Comparative Biochemistry and Physiology* **84A**, 483–486.